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Current strategies for cell delivery in cartilage and bone regeneration

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Several cell-based tissue-engineering therapies are emerging to regenerate damaged tissues. These strategies use autologous cells in combination with bioresorbable delivery materials. Major functions of a delivery scaffold are to provide initial mechanical stability, homogenous three-dimensional cell distribution, improved tissue differentiation, suitable handling and properties for delivery and fixation into patients. Delivery of cells can be achieved using injectable matrices, soft scaffolds, membranes, solid load-bearing scaffolds or immunoprotective macroencapsulation. Thus, to expand the clinical potential, next generation therapies will depend on smart delivery concepts that make use of the regenerative potential of stem cells, morphogenetic growth factors and biomimetic materials.

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Abbreviations
ECM extracellular matrix
MSC mesenchymal stem cell

Introduction

Strategies for engineering skeletal tissues are mainly focused on the restoration of pathologically altered structures based on the transplantation of cells in combination with supportive matrices and biomolecules [1,2]. This approach comprises the interactive triad of responsive cells, a supportive matrix template and bioactive molecules promoting differentiation and regeneration of the tissue structure [3,4]. A characteristic feature of all skeletal tissues is that their environment is rich in collagenous extracellular matrix (ECM), which is constantly remodeled by the cells. There are striking differences in the repair characteristics of bone, cartilage, ligament and intervertebral disc. In general, bone fractures are organized by the powerful regenerative capacity of the periosteum, but no comparable repair mechanism for eroded cartilage exists. Defects in cartilage penetrating in the subchondral bone are usually replaced by a mechanically inferior fibrous tissue formed by migrating marrow cells [5]. This tissue or partially filled superficial defect becomes the focus of progressive cartilage degeneration and destruction. In the case of intervertebral disc (another avascular tissue), regenerative potential is minimal and trauma to annulus fibrosus or nucleus pulposus invariably leads to degeneration.

In developing tissue-engineered constructs for articular cartilage, intervertebral disc and ligament, vascularisation can be neglected [6] as long as the construct volume to surface ratio allows sufficient nutrient supply by diffusion. Bone, however, cannot be produced as one piece in a size usually needed for transplantation. Therefore, diffusion can be facilitated by preformed vascular networks at the transplant site or by promoting vasculature within the construct using template design features or the slow release of angiogenic factors [7].

A critical requirement of skeletal tissue engineering is the design of specific biomaterials and scaffold structures. Biomaterial scaffolds control three-dimensional shape, guide tissue development and permit the convenient delivery of cells into patients. For cartilage, bone and intervertebral disc engineering a suitable biomaterial should provide or support initial mechanical stability, even cell distribution and good tissue biocompatibility [8]. In general, the amount of any biomaterial should be maintained at a minimum to avoid adverse effects such as accumulation of degradation products or inflammatory responses.

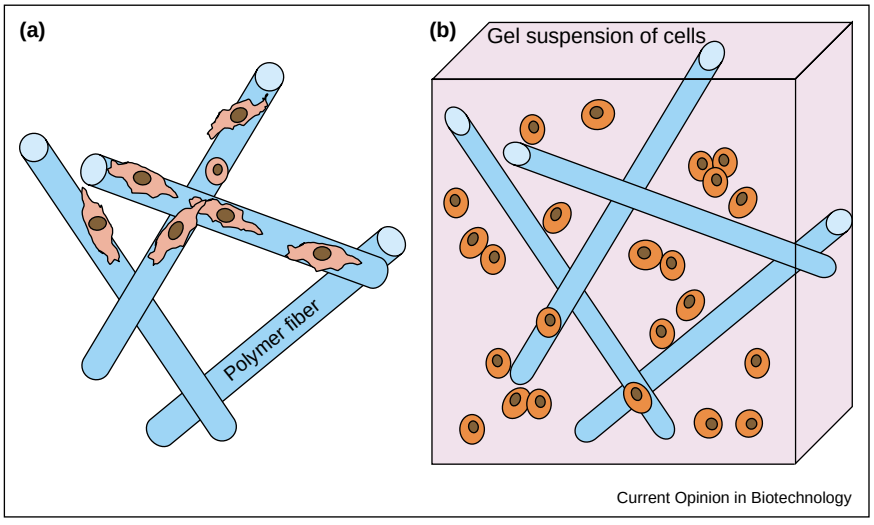
In this review, different functions of biomaterials for tissue engineering are explained and discussed with specific emphasis on the practical transfer of cultured cells or engineered tissues into the patient.

The functional role of biomaterials in skeletal tissue engineering

Adhesion substrate

Adhesion of cells is a basic process to stimulate proliferation *in vitro*. In tissue engineering, adhesion to the scaffold is important for containing cells within the delivery

Figure 1



Options for cell-seeding in scaffolds. **(a)** Cells are seeded onto the inner surface of the scaffold material. Cells attach and spread on the surface structures such as fibers. **(b)** Cells are distributed in the interconnecting cavities of a porous scaffold structure using a viscous embedding matrix component. Cells are not directly attached to the inner surface of the scaffold structure.

material (Figure 1). Similarly, attachment to surfaces activated with biomimetic peptides or growth factors induce cell differentiation and tissue maturation [9].

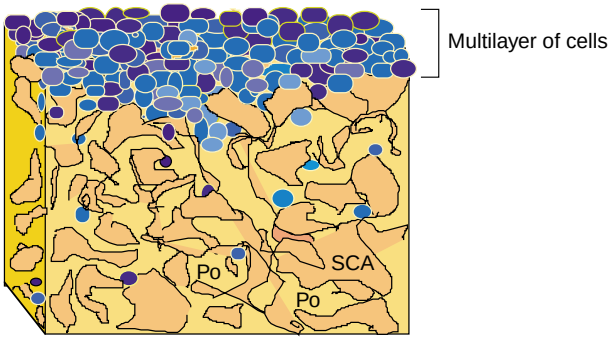
Mesenchymal cells such as chondrocytes undergo a process of phenotypic and functional dedifferentiation when expanded in a monolayer culture. By contrast, three-dimensional cell cultures provide the advantage of anchorage-independent cell growth, maintaining the differentiated phenotype that allows the synthesis of cell-specific pericellular or intercellular matrix [10]. It should be noted that the macromolecular assembly of newly synthesized collagens (heteropolymer formation) and proteoglycans is critical for tissue engineering, as this initially laden matrix serves as a template for subsequent matrix deposition and architecture [11]. Increasing evidence suggests that anchorage-independent growth in a semisolid medium is superior for chondrogenic differentiation of mesenchymal stem cells (MSCs). By contrast, osteogenic differentiation of cells is positively influenced by strong adhesion onto surfaces. Contrary to this notion, recent studies showed osteogenic differentiation of MSCs in hydrogel cultures without conventional methods of cell attachment on biomimetic surfaces [12].

Spatial distribution and guidance of cells

In skeletal tissues, cells are distributed within a dense ECM made up of collagens, proteoglycans, a complex mixture of phosphoproteins and other inorganic materials. For regeneration of these tissue types, the spatial distribution of cells in all areas of the repair tissue is preferred. While cell seeding on even surfaces is easy to perform, preparation of three-dimensional constructs is more difficult, depending on the matrix material used.

For even distribution, cells are mixed with polymer solutions that subsequently gel. Collagen, hyaluronate, fibrin, agarose, alginate and chitosan are frequently used embedding gels in tissue engineering applications. In contrast to gel immobilization, cell seeding onto porous membranes or solid scaffolds typically does not result in an even cell distribution. Sufficient pore size allow cells to migrate or adhere on the surface layer of a material, whereas interconnecting pores permit cells to grow into the interior region of the scaffold. Other technical challenges in using solid scaffolds include difficulties in viewing cells under the microscope during and after seeding and cell-multilayer formation on the scaffold surface owing to insufficient penetration (Figure 2). Moreover, few cells are retained within highly porous structures with large open pores. Therefore, scaffolds alone are not an ideal tool to provide homogeneous three-dimensional cell distribution and are largely considered as a means to provide early stabilization, ease of handling and delivery of cells. As a consequence, delivery materials with different properties can be used in combination to develop a suitable tissue-engineered construct; one example is the use of agarose with a mechanically rigid polymer fibre mesh [13]. Like uniform cell distribution, spatial guidance of cells is also necessary for the regenerative healing of tissue defects. In this respect, the use of cell constructs is again the preferred alternative over cells in a suspension without biomaterial component or even growth factor injection (Figure 3).

Figure 2



Conceptual drawing of cells (blue) seeded on a porous scaffold (SCA) such as collagen (orange). The cells can form multilayer cell sheets on the surface of the matrix and, in part, grow into the pores (Po) close to the surface. However, the limited size or interconnectivity of the pores usually inhibits cell distribution throughout the whole matrix construct.

Passive or active stimulation of tissue maturation

Cell differentiation and tissue maturation is one of the major goals in scaffold-based tissue engineering; therefore, in many cases biomaterials are designed to inhibit proliferation and stimulate differentiation of cells. Surface modifications to improve cell growth thus frequently underestimate this central theme in tissue engineering. In fact, the quality of cell adherence and cell spreading might not always reflect the differentiation status (e.g. passive differentiation of chondrocytes in agarose gels). Substrate microtopography is another important factor that influences cell behaviour; for example, osteoblasts adhere and proliferate well on smooth surfaces, but on microrough surfaces proliferation and attachment are reduced to favour differentiation [14•]. However, it is debatable as to whether cell differentiation and tissue maturation in skeletal tissue engineering can be achieved or controlled solely by choosing the appropriate physical and chemical surface properties of the carrier biomaterial. A biomimetic coating may be useful to 'activate' scaffolds with ECM molecules permitting cell migration, adhesion and differentiation [15,16]. It is likely that scaffolds modified with morphogenetic growth factors [17] and biomimetic molecules may not only support tissue formation as a 'smart' cell transplant, but might even open important perspectives to either reduce or even fully avoid the use of cultured cells in skeletal tissue engineering. Cell scaffolds could thus evolve as matrices containing complex cocktails of cell adhesions molecules and morphogenetic tissue factors [18••]. An important technical step in this direction has been recently proposed by Luo and Shoichet [19•] using photolabile hydrogels patterned with biochemical channels for guided cell growth.

Figure 3

The appearance of a tissue-engineered bone chip used for maxillary sinus augmentation. The construct contains gel-suspended mandibular periosteal cells loaded into resorbable fiber fleeces. Osteogenic conditions allow calcification *in vitro* before surgical transplantation. (Figure reproduced courtesy of Biotissue Technologies.)

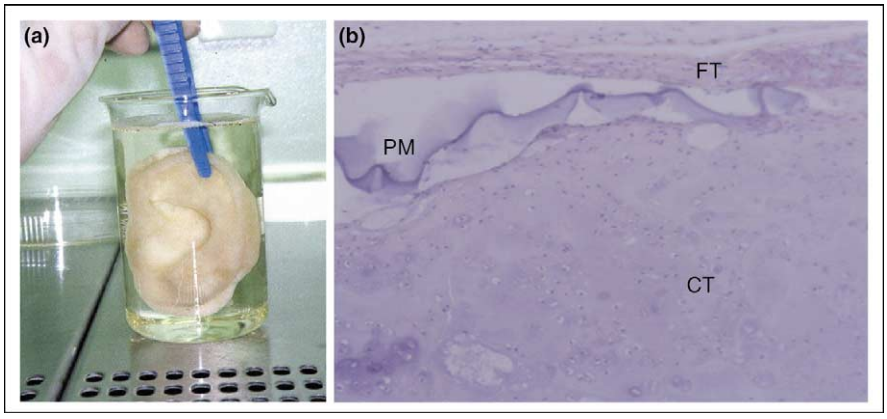
Protection of cells and shape of the tissue

Although tissue engineering has come close to producing successful therapies for the repair of joint cartilage, its utility in plastic surgery has so far had limited success. Severe cellular responses of the surrounding tissue strongly affect tissue formation and final shape. As a consequence, biomaterial coatings were proposed as a means to protect a maturing mesenchymal tissue from the potentially destructive environment *in vivo* [20]. Macroencapsulation of engineered cell transplants may allow sufficient consolidation of a newly formed ECM and could mimic the function of biological cell layers such as the perichondrium (Figure 4). Based on our current clinical understanding of engineered bone and cartilage transplants, it is likely that immune responses against even autologous cell constructs will become a significant obstacle in the development of future cell-based therapies. This might not just include potential responses against the applied biomaterials or their degradation products, but also the possible response against molecules presented by culture-expanded or genetically altered cells or their ECM constituents. Therefore, the development of biomaterials for the temporary encapsulation or protection of cell constructs for delivery into patients could be important for successful cell-based strategies of the future.

Handling of cells and delivery into the patient

For clinical application of engineered tissues, convenience in surgical handling is important. Delivery of cells by

Figure 4



Macroencapsulation of cartilage transplants for immune protection. **(a)** Tissue-engineered cartilage in the shape of a human ear is encapsulated by a polyelectrolyte complex membrane composed of sodium cellulose sulfate as a polyanion and polydiallyldimethylammoniumchloride as polycation. **(b)** Histological appearance of the macroencapsulated tissue showing a clear separation of tissue engineered cartilage (CT) from surrounding fibrotic tissue (FT) due to the polyelectrolyte membrane (PM).

injection or minimally invasive surgery is thus a preferred method. By contrast, where engineered tissues compete with solid ceramics, metals or plastics for replacement, initial geometrical configuration and mechanical stability is critical. Presently, it is not possible to engineer bone or cartilage tissue *in vitro* using soft matrices that are comparable in mechanical stability to native bone or cartilage. Such immature and often fragile cell constructs (e.g. constructs based on fibrin or collagen alone) might not meet the usual expectations of surgeons, although the transplant may develop into a mature tissue with time after transplantation. Likewise, bone cell transplants based on soft biomaterials may not be visible on the X-ray immediately after implantation and complicate follow up procedures. Therefore, handling and delivery of cell constructs must be convenient, efficient and reliable under the standard conditions in an operating room. To achieve this goal, an interdisciplinary team of surgeons, biologists and biomaterial scientists need to work together to ensure both the biological quality as well as clinical and practical acceptance of the engineered tissue.

Strategies for cell delivery in skeletal tissue engineering

Injectable delivery

Despite numerous studies on biomaterials for tissue reconstruction, fewer efforts have been made to develop injectable delivery strategies for skeletal tissue repair. The transplantation of autologous chondrocytes is still considered to be one of the major clinical applications of skeletal tissue engineering [21]. Carticel, a product developed by Genzyme Biosurgery (http://www.genzymebiosurgery.com), offers easy handling of chondrocytes as they are provided as an injectable liquid suspension. However, the need for invasive surgery and the difficult suturing technique of the periosteal flap complicates this otherwise simple delivery concept. Such difficulties point to the importance of cell delivery in a minimally invasive manner. To treat irregularly shaped joint defects, injectable cell delivery systems offering early mechanical stabilization by *in situ* polymerization have been designed [22,23]. Thermoreversible, *in situ* gelling, biocompatible formulations have been tested for chondrocyte delivery [24].

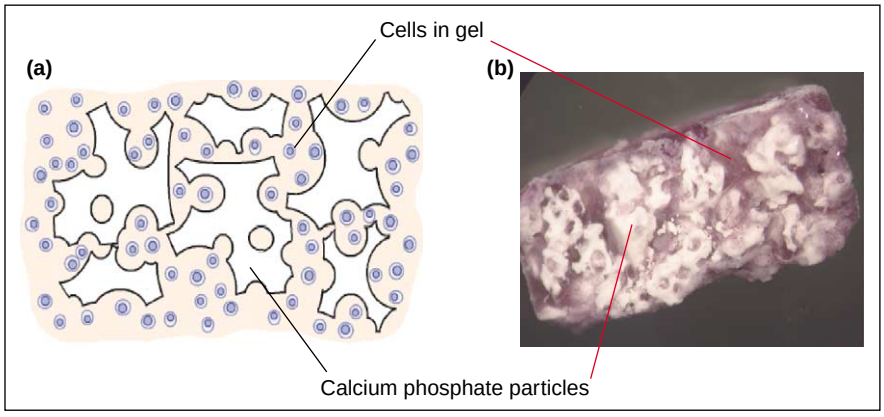
Recent studies have evaluated the suitability of injectable carriers to support MSC function [25]. For joint cartilage repair, however, it is presently uncertain as to whether injections of MSCs into the joint cavity can be directed into regenerative healing and produce significant clinical improvement for the patient [26]. It should be noted that so far chondrocyte transplantation into sites other than articular defect has been unsuccessful in immunocompetent animals.

In the case of irregular bone defects resulting from the surgical removal of cysts or tumours, injectable bone-forming cell-matrix composites might be a more appropriate method than conventional scaffolds. Early stabilization could be achieved using injectable gels (fibrin or collagen) in combination with tricalcium phosphate or hydroxyapatite particles [27] (Figure 5). Likewise, if cell anchorage is preferred before *in vivo* delivery, injectable carrier beads with attached cells can be used [28].

Delivery with soft scaffolds

If the injectable delivery of cells is not feasible to retain cells in the repair site, soft scaffolds such as porous membranes or fibrous structures are alternate options. These materials are convenient for holding cells in place during surgical handling, fixation and initial tissue repair. As a requirement, these materials should be resilient to rupture, possess sufficient tensile strength and resist

Figure 5



Engineered vital bone paste based on calcium phosphate particles and gel-suspended bone-progenitor cells. **(a)** Unregular shaped particles form interconnecting macropores to trap suspended cells. **(b)** Staining of metabolically active cells in the pores. (Figure reproduced courtesy of Biotissue Technologies.)

stress from the use of surgical instruments (squeezing and bending etc.). Textile materials have proved robust in handling engineered cartilage transplants. Their tensile strength may well be adapted to engineer tendons and ligaments. Scaffolds need not necessarily be seeded with cells to benefit from this handling advantage. Often, cell suspensions prepared in gels can be stabilized with a mechanically stable solid scaffold to achieve convenient tensile strength, pressure resistance or elasticity. Several recent studies have used this concept in clinical applications of tissue-engineered bone and cartilage transplants [29•,30,31•,32].

Load-bearing cell-seeded replacement materials

Another method of delivery is the use of solid scaffolds seeded with cells. Following implantation, these scaffold–cell constructs stimulate the formation of repair tissue while maintaining adequate integrity. The degradation rate of the scaffold can be attuned so that sufficient strength and stiffness is maintained as a gradual shift of mechanical load takes place from the scaffold to the strengthening, maturing tissue. Increased mechanical functions of the scaffold should also be balanced with the interconnecting porosity. This facilitates nutrient supply into central regions of engineered transplants preventing adverse effects such as tissue necrosis [33•]. With the currently available engineered bone implants, cells receive their nutrition through diffusion before new blood vessels can be formed to support tissue formation. As a consequence, the possible geometries and dimensions of tissue-engineered bone for orthopedic applications are still limited.

Delivery of moulded transplants

In the plastic reconstruction of tissues such as ear and nose cartilage, the natural three-dimensional shape of the tissue needs to be achieved. Current techniques for the manufacture of three-dimensional cell constructs have made it possible to design engineered cartilage in the shape of an ear or nose. The typical ear-shape is either achieved with an ear-shaped cell-free template material [34] or a cell–scaffold construct is moulded using a silicon template before three-dimensional cell culture or transplantation [35].

Fabrication of a custom-designed scaffold for an individual patient depends on an appropriate collection of imaging data about the exact dimensions of the tissue defect and can be cumbersome. However, emerging rapid prototyping (RP) technology has demonstrated its usefulness to design the custom-made moulds that are specifically required to treat a certain shaped bone or cartilage lesion [36]. An alternative is to manufacture a set of frequently used shapes of the scaffolds by RP and use them off the shelf.

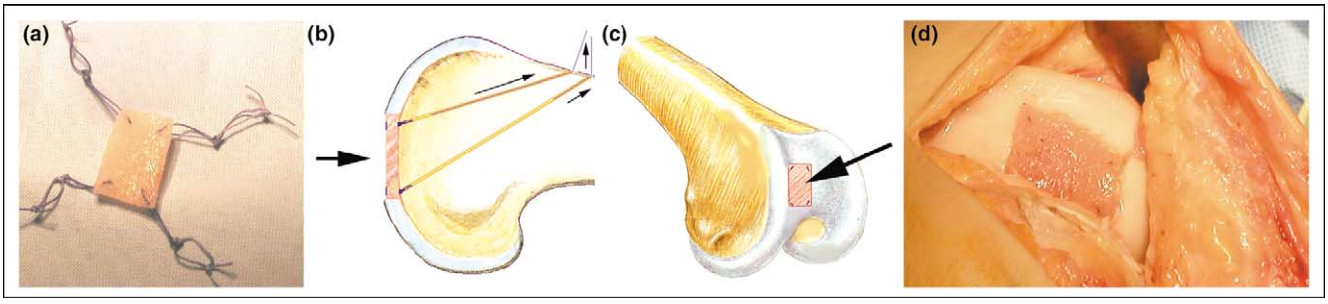
Encapsulation for immunoprotection

Macroencapsulation with a polyelectrolyte membrane was proposed earlier as a possible method to stabilize engineered tissues in plastic reconstructive surgery [20]. Supporting this notion, Kamil *et al.* [37] showed recently that chondrocytes in a perforated hollow gold mould filled with chondrocyte constructs allowed auricular-shaped cartilage formation in immunocompetent animals, which is far more difficult than cartilage formation in a nude mouse. It is hypothesized that the implanted mould may have protected the engineered tissue *in vivo*.

Use of shape memory constructs for minimally invasive delivery

Except for injectables and some soft scaffolds, the delivery of cell constructs requires open surgery. Shape

Figure 6



Anchorage of a tissue-engineered cartilage transplant consisting of a chondrocyte gel suspension in a resorbable polymer fleece scaffold (BioSeed®-C). **(a)** Preparation double-loop knots with resorbable thread. **(b,c)** Transosseous fixation at four corners with the aid of Kirschner wires. **(d)** Appearance of tissue-engineered cartilage transplant after surgical fixation. (Figure reproduced courtesy of C Erggelet, University of Freiburg, Germany.)

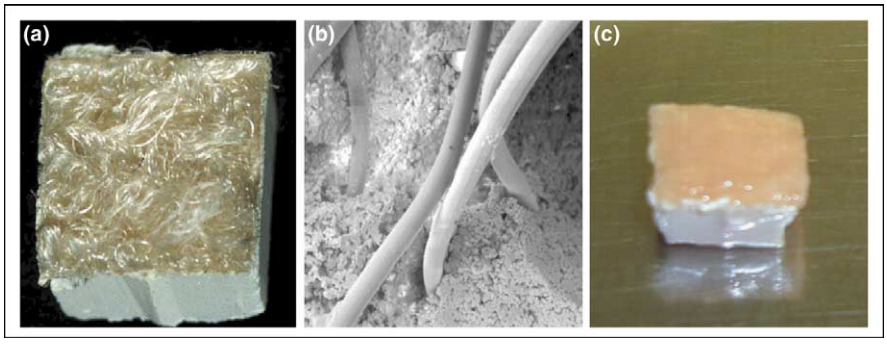
memory materials offer a fascinating perspective to deliver bulky scaffolds via minimally invasive surgery. Using these smart materials future scaffolds may be fabricated in a condensed state before transplantation, which then later acquires the desired shape *in vivo*. For example, degradable thermoplastic polymers that change their shape with increasing temperature might be used for this purpose [38••]. In a recent study, macroporous alginate hydrogels were compressed to smaller dimensions and later rehydrated with cell suspensions *in vivo* to recover their original size [39].

Fixation of transplants

The fixation and anchorage of engineered tissues at a repair site is rarely discussed in the published literature on biomaterials used for skeletal tissue engineering. It is now understood that surgical fixation of engineered transplants is a crucial step for successful cell-based therapy. For this purpose, cell transplants should be suitable for suturing, gluing, pinning, and press-fitting of transplants into accurately prepared cylindric defects. In theory, the artificially grown cartilage layers could be attached directly to the defect joint surface using fibrin glue or could be fixed using resorbable pins. For cartilage transplants it was shown that a four-point transosseous suture fixation is possible and also allows arthroscopic delivery [29•] (Figure 6). Other matrix-based cartilage transplants are fixed with fibrin glue or could be applied by press-fitting techniques [40,41].

Another strategy that has gained wide attention is the development of an osseo-integrating interface within the cartilage implant using either a calcium carbonate or hydroxyapatite/tricalcium phosphate scaffold [42,43]. Similarly, recently proposed engineered osteochondral transplants with an engineered osteoblast layer might be suitable for press-fitting implantation in a similar manner to the existing osteochondral mosaic arthroplasty [8,44–46] (Figure 7). The ultimate aim of these strategies is to achieve a permanent, solid connection between

Figure 7



Biphasic composite graft of non-woven fibers and calcium phosphate for osteochondral cartilage repair. **(a)** Fiber mesh is fixed on calcium phosphate bone cement. **(b)** Resorbable fibers are incorporated into the underlying cement. **(c)** Appearance after chondrocyte suspension is cultured in the composite graft.

implant and the host tissue. This will become increasingly important once there is more clinical experience with tissue-engineering products.

Conclusions

When biomaterials are evaluated for their use in tissue engineering one has to be able to differentiate between specific requirements for handling and application of the construct and those for cell behaviour such as differentiation or proliferation. Tools for cell handling and delivery depend primarily on the clinical application and may or may not be distinct from strategies that promote tissue formation. The development of a cell-based therapy in skeletal tissue engineering might start with defining the most convenient delivery concept for handling and application for the surgeon and could then be adjusted or supplemented with biocompatible materials for best possible tissue maturation and biocompatibility.

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